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BIOLOGICAL BULLETIN

THE INFLUENCE OF THE NUCLEUS ON THE BEHAVIOR OF AMŒBA.[1]

H. S. WILLIS.

INTRODUCTION.

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In the course of experimental work on *Amœba* in November, 1914, marked differences were observed in the behavior of the different parts of specimens cut in two. At the suggestion of Dr. S. O. Mast, an attempt has been made to ascertain whether these differences depend upon the nucleus and, if so, in what respects. I wish here to acknowledge my great indebtedness to Dr. Mast for his helpful and constructive suggestions and for his kindness in supervising both the experimental work and the writing of this paper.

[1] From the Zoölogical laboratory of the Johns Hopkins University.

Gruber (1912) found that parts of *Amœba proteus* containing a nucleus behave very much like normal specimens, but that parts without a nucleus behave very differently; yet, in spite of this, he held that the nucleus in general has no influence upon protoplasmic movement. Hofer (1890) in observations on the same species, obtained results similar to those of Gruber. He asserts (p. 118) that movements in the parts with the nucleus are similar to those in normal specimens, as are also those in the other parts for a period of 15–30 minutes after division, but that later the movements in the parts without a nucleus differ from those in normal specimens in rate of locomotion, in regularity of movement, and in the number and length of pseudopods.

Hofer holds that these differences might be considered to be due either to the injury received during the operation or to the influence of the nucleus. But he maintains that, since any injury sustained from the operation affects both fragments alike, the operation could not be considered the cause of the observed difference in behavior. He consequently concludes that the real cause is to be found in the nucleus. He holds, however, that the influence of the nucleus may be conceived to be direct or indirect; that is, that behavior may be due to an impairing of the elementary functions (such as digestion, respiration, and excretion) controlled by the nucleus. But Hofer found that the process of digestion in the absence of the nucleus continues for several days after division; that respiration takes place in the absence of the nucleus; and that excretory functions in enucleated segments continue till death. He therefore comes to the conclusion that the nucleus secretes a chemical substance and that behavior is controlled through this. He thinks a certain amount of this substance is stored up in the different parts of the protoplasm, and that the normal movement of the enucleated segments for 15–30 minutes after the operation is due to the influence of the substance thus stored. Hofer maintains that, since movement occurs in parts without a nucleus, cytoplasm has the power of movement; but since the movement in these parts is more irregular and haphazard than it is in nucleated parts, he holds that the nucleus must have a regulatory function. In other words, he thinks the nucleus serves as a regulatory "centrum" for behavior.

Verworn (1909) agrees with Hofer in holding that there is a difference in the behavior of parts of *Amœba* with and parts without a nucleus, but does not agree with him in the conclusion that the nucleus exerts a direct influence on the movement; that is, he does not think that the nucleus is a regulatory "centrum" for movement.

Judging from the results of my experiments, it is clear that there are distinct differences in the behavior of parts of *Amœba* which contain and parts which do not contain a nucleus. Differences in such parts have been observed in the character of movement, in the accuracy of orientation in light and in the rate of locomotion. It is clear, also, that these differences are traceable to the nucleus.

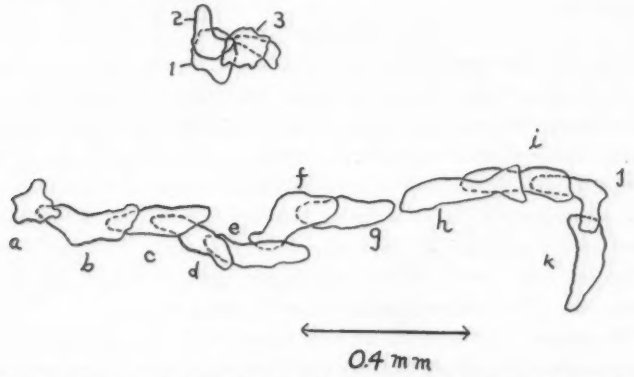


FIG. 1. Camera sketches showing changes in form and the characteristic movements in the nucleated and enucleated parts of an amœba immediately after being cut in two. The arrows indicate the direction of movement. *1–3*, enucleated part; *a–k*, nucleated part; *mm*, projected scale. The sketches of the enucleated part were made at five-minute intervals; those of the nucleated part at one minute intervals. Note that the nucleated part progressed regularly in a given direction, and that the enucleated part changed its form and position only slightly.

MATERIAL AND METHODS.

The specimens used for the most part in this work appeared in a battery jar containing an old paramecium culture. They corresponded to descriptions of *Amœba proteus* and were rather sensitive to light. Nucleated and enucleated parts were obtained by cutting specimens in two with finely drawn glass rods.

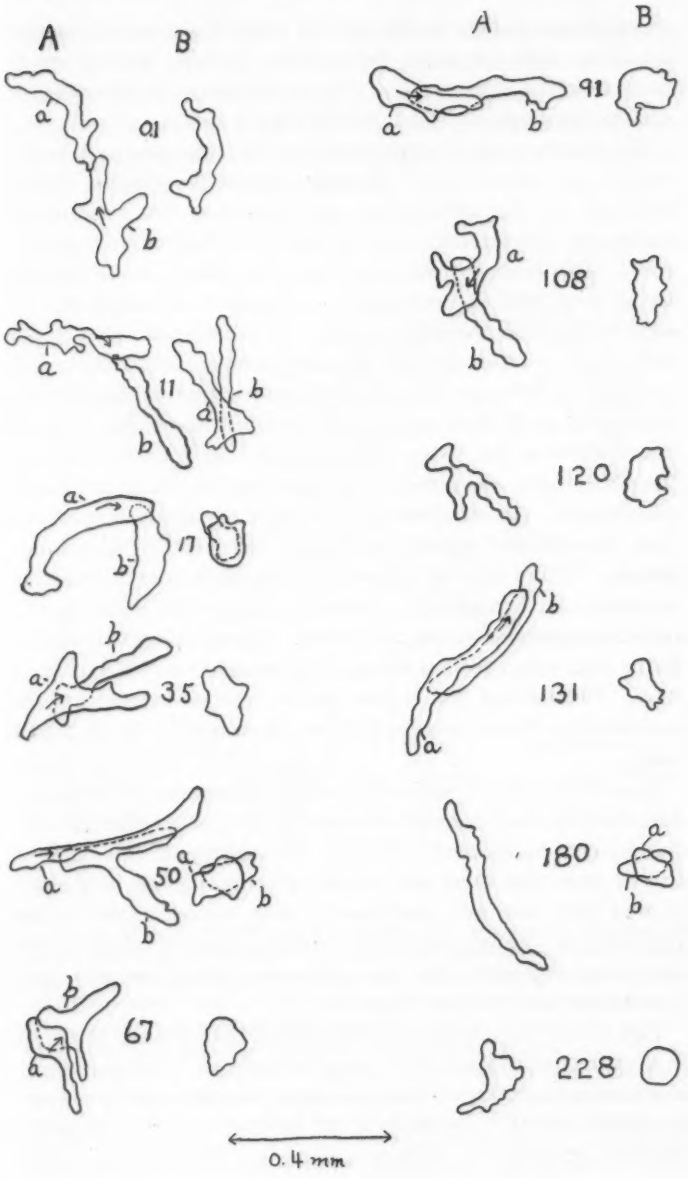
The cutting was done under a binocular. The parts of each individual were usually enclosed under a cover-glass by means of a ridge of vaseline so applied along the edge as to support the cover-glass and prevent drying. Thus the parts had entire freedom of movement and both were continuously subjected to the same environment; and, under these conditions, they were studied, observations being made both with a compound microscope and a binocular. Under the sealed cover-glasses the divided amœbæ did remarkably well, the nucleated parts living on an average of approximately ten days, *i. e.*, practically as long as normal specimens under the same conditions, and the enucleated parts about half as long. In the following descriptions, the two parts will frequently be designated fragments or segments.

Movement.

Normal Specimens.—In the process of locomotion in normal individuals of the species studied, pseudopods usually appear alternately on the two sides of the organism near the anterior end. A pseudopod appears, for instance, on the right, elongates and enlarges by the flow of protoplasm into it until it constitutes the main portion of the animal; then from this there is formed a new pseudopod on the left side. This, in turn, elongates and enlarges, after which a new pseudopod again forms on the right side, etc. Thus the organism takes a zigzag course.

Fragments.—In general it was found that the movement in fragments containing a nucleus is substantially like the movement exhibited in normal specimens, and, in some instances, for a period of 10–20 minutes after division, it was found to be similar in enucleated parts. Usually, however, the movement is strikingly different in such parts, it being slow and irregular, and frequently accompanied by contractions. The pseudopods, all

Fig. 2. Series of camera sketches illustrating the difference in the movement of nucleated and enucleated segments of *Amœba*. The former is shown in columns A, the latter in columns B. The numerals between the columns indicate approximately the intervals of time, in hours, between the cutting of the amœba and the production of the adjoining sketches in the two columns. The two sketches, *a* and *b*, in every case were made about one minute apart. They show the changes in position and in form of the segments during this time. Whenever there is but one sketch opposite the numerals, it indicates that there was no change in the organism. *a*, first position; *b*, second position, arrows, direction of movement, *mm*, projected scale.


A
B
a
b
91
108
11
120
17
131
35
180
50
228
67
0.4 mm

of which are usually small, are not formed alternately as they are in the parts containing the nucleus. Neither do they always occur near the anterior end. These differences in movement in the two segments are illustrated in Figs. 1 and 2.

The nucleated part represented in Fig. 1 formed pseudopods within one minute after division. At three minutes it was attached to the substratum and exhibited the coördinated movement characteristic of a normal individual (Fig. 1, *a*, *b*, *c*, etc.). This fragment was observed every three to five minutes for an hour, and the movement was found to be essentially the same throughout the entire period. The enucleated part of this individual on the contrary, became globular immediately and remained so for approximately five minutes during which time a number of small short pseudopods were directed outwards in all directions from the body. These became fewer and larger, and the entire body elongated in the direction of one of the larger pseudopods. An attachment of the body to the substratum was then formed and regular movement followed for about one minute. There was no subsequent locomotion or "streaming movement"; the organism, however, changed its shape by frequent contractions of the cytoplasm. These changes continued for an hour when the experiment was brought to a close (Fig. 1, 1–3). Throughout the entire period, the movement in the enucleated part was very much slower than that in the nucleated part.

Essentially all of these characteristic differences in the movements of the two fragments in question are further elucidated in the sketches reproduced in Fig. 2. By referring to this figure it will be seen that there was considerable locomotion in the nucleated part and that pseudopods were formed more or less regularly on the opposite sides of the segment; while in the enucleated segment there was extremely little locomotion and pseudopods were formed irregularly.

Figs. 1 and 2 are typical illustrations of the behavior observed in all of the specimens studied—forty in number. The movement in all nucleated parts of these specimens was like that in normal specimens and the movement in all enucleated parts was quite different from that in normal specimens. The behavior of 17

pairs of these parts taken at random is briefly summarized in Table I. By referring to this table, in which observations on locomotion are recorded for 13 of the 17 individuals, it will be seen that locomotion occurred in 13 of the nucleated parts, while there was no locomotion in the enucleated parts. Other matters in this table will be considered later.

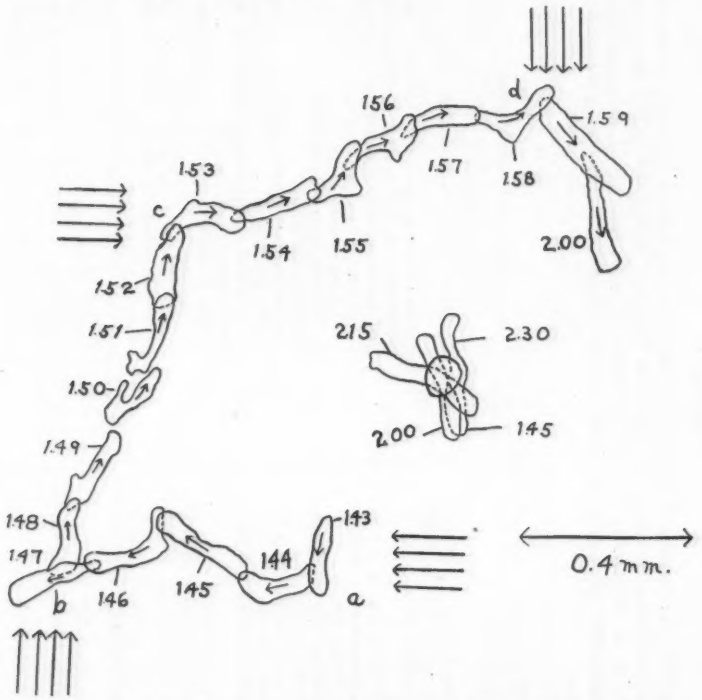


FIG. 3. A series of camera sketches showing the reactions of a nucleated and an enucleated part of an amœba in a horizontal beam of light. *a–d*, nucleated part; 1.45–2.30 enucleated part; large arrows, beam of light; small arrows, direction of movement; 1.43–2.00, time at which sketches of nucleated part were made; 1.45–2.30, time at which sketches of enucleated part were made; *mm.*, projected scale. Note that the nucleated segment oriented fairly definitely; at *a* (1.43) the rays of light were at right angles to the moving segment; at 1.44 the segment had turned and become directed from the source of light. A similar response occurred at *b*, *c*, and *d* after the direction of the rays of light had been changed in each case. Note also that the enucleated part did not orient in the light. Both parts were continuously in the same field and were subjected to the same changes in illumination.

TABLE I.

Table showing the relation between the reactions of fragments of *Amœba* and the presence or absence of the nucleus; the position the fragments occupied in the intact specimens; the size of the fragments; the length of time the various pairs of fragments were studied, and the attachment of the parts to the substratum. Note that the nucleated parts, regardless of size or position, remained attached to the substratum and responded essentially like normal specimens, and that the enucleated parts rarely, if ever, became attached and did not respond like normal specimens.

Designation of the Individuals Cut into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
	Nucleus.	Anterior end.	Slightly larger.	¾	Part attached 1 min. after division.	Locomotion.
1	No nucleus.	Posterior end.			Attached one min. after division. Attachment lost after 5 mins. Regained and lost in first 20 min. after division.	Slight motion.
2	Nucleus.	Anterior end.	Larger.	½	Attached after 2 min.	Locomotion.
	No nucleus.	Posterior end.			Did not attach.	Became spherical.
3	Nucleus.	Anterior end.	Equal.	1/6	Attached soon after division.	Locomotion.
	No nucleus.	Posterior end.			Attached slightly.	No locomotion.
4	Nucleus.	Posterior end.	Equal.	1/5	Attached 2 min. after division.	
	No nucleus.	Anterior end.			Did not attach.	
5	Nucleus.	Anterior end.	Equal.	¼	Attached 2 min. after division and remained so.	
	No nucleus.	Posterior end.			Did not attach.	
6	Nucleus.	Posterior end.	Equal.	1 ⅓	Attached.	Movement normal.
	No nucleus.	Anterior end.	Equal.		Attached 30 min. after division. Attachment broken by gentle blowing on the water. Did not attach again.	Irregular motion.
7	Nucleus.	Anterior end.	Larger.	1	Attached 7 min. after division and remained so.	Movement normal.
	No nucleus.	Posterior end.			Did not attach.	Became spherical.
8	Nucleus.	Anterior end.	⅓ larger.	1	Attached 3 min. after division and remained so.	Movement normal.
	No nucleus.	Posterior end.			Attached slightly 25 min. after division; lost at 30 min. No further attachment.	Pseudopods irregularly formed.

TABLE I.—*Continued.*

Designation of the Individuals Cut Into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
9	Nucleus.	Posterior end.	Equal.	1	Attached 2 min. after cutting. Attachment broken 5 min. later. Attached again in 1 min. and remained so.	Locomotion.
	No nucleus.	Anterior end.			Attached 2 min. after division. Unattached 5 min. later. Attached at 20 min. after division. Unattached at 25 min. and remained so.	Irregular motion.
10	Nucleus.	Anterior end.	Smaller.	1	Did not attach for an hour.	
	No nucleus.	Posterior end.			Did not attach for an hour.	
11	Nucleus.	Posterior end.	Equal.	1½	Remained attached after first 10 min.	
	No nucleus.	Anterior end.			Unattached for 1 hour when it slightly attached. Remained attached 15 min. No further attachment.	
12	Nucleus.	Posterior end.	Larger.	2½	Attached 5 min. after cutting. Attachment broken 3 min. later. Attached again 10 min. after division, and remained so.	Locomotion.
	No nucleus.	Anterior end.			Attached 15 min. after division. Attachment broken 3 min. later. It did not attach again.	
13	Nucleus.	Anterior end.	Equal.	3	Attached 5 min. after division. Attachment broken 3 min. later. Formed again after 2 min. and remained so.	Locomotion.
	No nucleus.	Posterior end.			Attached 5 min. after division. Attachment broken 3 min. later; then formed again after 20 min. This was lost 2 min. later. No further attachment.	Irregular motion.

TABLE I.—*Continued.*

Designation of the Individuals Cut into Two Parts.	Contents of Parts.	Position Occupied by the Two Parts in Intact Amœba.	Relative Size of Parts.	Length of Time Studied (in Hours).	Relation of Parts to Substratum.	Behavior.
14	Nucleus.	Anterior end.	⅓ larger.	3	Attached 4 min. after cutting and remained so.	Locomotion.
	No nucleus.	Posterior end.			Attached 1 min. after division. Unattached at 5 min. after. 15 min. later attached again for several min. No subsequent attachment.	Much contraction.
15	Nucleus.	Posterior end.	Smaller.	52	Attached 5 min. after division. Attachment was noticed at many observations during the 52 hours.	Locomotion.
	No nucleus.	Anterior end.			Observed at intervals of 5 min. each for a few hours, then observed every few hours. No attachment.	Contractions.
16	Nucleus.	Posterior end.	Equal.	72	Attached 10 min. after division and remained so.	Locomotion.
	No nucleus.	Anterior end.			No attachment.	No locomotion.
17	Nucleus.	Posterior end.	Equal.	72	Remained attached after 1 min. following division.	Locomotion.
	No nucleus.	Anterior end.			Attached 5 min. after division. Remained attached for ½ hour. No further attachment.	Contractions.

It has been thus shown that there is a difference in the general movements of the segments containing and those not containing a nucleus. Attention is now directed to the reactions to light in such segments, especially the reactions resulting in orientation.

Orientation in Light.

Normal Specimens.—It is well known that certain species of *Amœba* when placed in a horizontal beam of light, usually turn until they are directed from the source of light and then continue in a fairly direct course; that is, they orient and are negative. If now the position of the source of stimulation is changed so as to illuminate the organisms from the side, they again turn until they are directed from the light, that is, they reorient. In the form under consideration the reactions to light are marked and orientation is fairly precise.

Fragments.—Fragments containing a nucleus present precisely the same kind of reaction as do normal specimens when subjected to similar light conditions. These parts react readily to light; the movement is rapid; and the process of orientation is very much like that found in intact specimens. On the other hand, enucleated parts usually give no evidence whatever of orientation. Experiments were made on 25 pairs of segments. In 21 of these the nucleated parts oriented approximately as precisely as normal individuals, but in the enucleated parts no indication of orientation whatever was observed except in three instances, and in these there was only a mere suggestion of orientation. This will be described in more detail later.

Typical of the reactions in the 25 experiments mentioned above are the responses to light of the parts shown in Fig. 3. The reactions here represented, however, are somewhat more exact and definite than those observed in some of the other segments. In this case the enucleated fragment was the larger of the two and contained the contractile vacuole. Both segments were kept under the same cover-glass and were subjected to the same light conditions. A 165-C. P. tungsten lamp was placed 13 cm. distant from the reacting segment, and a piece of colorless glass absorbed the heat waves from the lamp. A compound microscope and a camera lucida were used. The temperature at the

microscope was 29.5° C. The results obtained are in part recorded in Fig. 3. It may be observed from this figure that the nucleated part moved from the light and that with each change in the direction of the rays of light, there was a corresponding change in the direction of the movement of the segment, while on the other hand, there was no indication of orientation in the enucleated fragment. The two parts were subjected to precisely the same conditions throughout the entire experiment.

As previously stated, there was usually no indication whatever of orientation in enucleated segments, and it is questionable whether any of these parts actually oriented, yet, in a number of cases, slight movement from the light occurred, and in three cases there was a change in the direction of movement with a change in the direction of the light. This movement from the light may have been a reaction to the light in the form of orientation or it may have been merely an accidental movement made without regard to the light. The facts regarding the three cases are these. One of the segments, while moving at right angles to the light, formed a pseudopod on the shaded side, and movement occurred in the direction of the pseudopod. With a change in the direction of the light, a short pseudopod was formed again on the shaded side, but at this point the fragment assumed a globular shape. In another segment movement occurred for several seconds at right angles to a horizontal beam of light, then the direction was changed, and the fragment moved from the source of stimulation. Upon a change in the direction of the light, this fragment changed the direction of its course and again moved from the light for approximately one minute and then became globular. In the third enucleated segment substantially the same reaction was observed. If these fragments actually oriented in response to the light, the process of orientation in them was essentially different from that in nucleated fragments.

There is, therefore, a difference in nucleated and enucleated parts of *Amœba* in their response to light as well as in the character of their movements; but there is also a difference in the rate of locomotion as will be demonstrated presently.

Rate of Locomotion.

Normal Specimens.—The rate of locomotion varies greatly in different individuals. Some specimens move nearly twice as fast as others. Quite a number of individuals were observed to move rather consistently at 0.27–0.3 mm. per minute; others moved at an average rate of 0.12–0.15 mm. per minute. There is a fairly definite relation between the size of the moving organism and the rate of movement. An animal travels a distance that is approximately two thirds the length of its body in one minute. The actual distance traveled by a large animal is greater for a given length of time than the actual distance traveled by a small animal for the same length of time under the same conditions. This is clearly demonstrated by the results recorded in Table II. By referring to this table it will be seen that the ratio of the distance traveled per minute to the length of the body of the specimen is approximately the same in all cases.

Table II.

Relation between size of *Amœba* and rate of locomotion. The average rate of locomotion was computed from five readings extending one minute each. The average length was computed from five measurements of the moving organism at intervals of one minute each. The rate was measured by projecting the moving organism on a scale with a camera lucida.

Maximum Length of Specimens Observed.	Average Distance Traveled per Minute.	Ratio of Distance Traveled per Minute to Length of Body.
0.36 mm.	0.24 mm.	0.66
0.34 "	0.20 "	0.59
0.35 "	0.23 "	0.65
0.37 "	0.24 "	0.65
0.21 "	0.15 "	0.71
0.26 "	0.17 "	0.64
0.20 "	0.14 "	0.72
0.26 "	0.17 "	0.64

Fragments.—In the work on the rate of locomotion in fragments, the two parts were on an average approximately the same size. Observations were made on each pair of segments at about the same time. Both were kept under the same cover-glass and in the same environment. It was found that the rate of movement in nucleated fragments, like that in normal specimens, bears a definite relation to the size of the body. Large nucleated

segments usually travel greater distances in a given length of time than do small ones. This is shown in the results recorded in Table III. In this table are given the lengths of four intact specimens together with the rate of locomotion per minute, and the length of body, and the rate of locomotion in the nucleated and enucleated parts cut from the four intact specimens.

TABLE III.

Relation between rate of locomotion and length of body in four intact specimens of *Amœba* and in the nucleated and enucleated parts of these four specimens.

Normal Specimen.		Nucleated Part.		Enucleated Part.	
Average Length of Body.	Rate of Locomotion per Minute.	Average Length of Body.	Rate of Locomotion per Minute.	Average Length of Body.	Rate of Locomotion per Minute.
.376 mm.	.244 mm.	.275 mm.	.150 mm.	globular	negligible
.355 "	.230 "	.210 "	.150 "		
.340 "	.200 "	.220 "	.140 "	.230 mm.	?
.360 "	.240 "	.200 "	.128 "	.230 "	.074 mm.

The table shows that the larger intact specimens and the larger nucleated parts travel greater distances per minute than do the smaller ones. It also shows that locomotion in the enucleated parts is very much slower than it is in the nucleated parts. This is, however, more clearly shown in Table IV in

TABLE IV.

A comparison of the rate of locomotion in millimeters per minute in the nucleated and the enucleated parts of ten different individuals.

Nucleated Parts.	Enucleated Parts.
0.15	0.002
0.13	0.074
0.13	0.010
0.18	0.020
0.13	0.008
0.15	0.110
0.16	0.000
0.19	0.006
0.17	0.006
0.13	0.004

which the rate of locomotion in nucleated and enucleated segments of the same individual is compared. Each number in the two columns of this table represents the average rate of

locomotion for five trials, each continuing for one minute. The figures in the left column show the rate of locomotion in the nucleated parts; those figures immediately opposite, in the other column, show the rate of locomotion in enucleated parts. For instance, the nucleated segment of the individual first cut moved in five trials at an average rate of 0.15 mm. per minute, and the enucleated part cut from the same individual moved at an average rate of 0.002 mm. per minute. From the table one thing is strikingly evident: movement in nucleated parts is always much more rapid than that in enucleated parts; in no case does the rate of locomotion in parts without a nucleus equal that in parts with a nucleus.

Possible Causes of Differences in Behavior.

From the experiments cited above, it is evident that there are differences in the behavior of parts of *Amœba* with a nucleus and parts without a nucleus; differences in the character of movement, in orientation, and in the rate of locomotion. Now, to what may these differences be attributed? The two parts differed in size, in their position in the amœba cut to produce them, in the possession of a contractile vacuole and a nucleus. The difference observed in their behavior must be ascribed to some of these differences in structure.

Size.—In this work nearly 125 amœbæ were cut, each into two parts, one of which contained a nucleus. In approximately one half of these cases, the part without a nucleus was as large as or larger than the part with a nucleus. Records of the two parts of 13 of these amœbæ, taken at random, may be found in Table I. Both parts in all cases were kept under the same cover-glass and were subjected to the same or similar conditions. All of the nucleated parts behaved like normal specimens, but none of the enucleated parts, regardless of the relative size of the two parts. It appears, therefore, that differences in the size of parts do not determine the differences in behavior.

Contractile Vacuole.—The contractile vacuole was present in the nucleated parts of the 125 specimens experimented upon about as often as it was in the enucleated parts. There was no observable difference in behavior associated with the presence or

absence of the vacuole. Enucleated parts with the vacuole apeared to behave in every way similarly to enucleated parts without a vacuole. The same is true for nucleated parts. The vacuole, therefore, cannot be reckoned as a determining factor in the behavior of the fragments.

Position Occupied by Fragments in the Intact Amœba.—When an amœba is cut into two parts, the part containing the nucleus may be from what was the anterior end or the posterior end of the original specimen. The behavior was carefully studied in 25 parts, 13 from the anterior and 12 from the posterior end of the intact individual. The results obtained are recorded in Table I. No evidence was obtained indicating that the reaction of the parts depends upon their location in the specimens from which they were taken. The nucleated parts from the anterior end responded precisely like those from the posterior end. Obviously, then, the position in the intact specimen is not a determining factor in the behavior of fragments.

The Nucleus.—We have demonstrated that parts of *Amœba* which contain a nucleus behave essentially like normal specimens, while those which do not contain a nucleus behave quite differently, and that this difference in the behavior of the parts is dependent upon neither their relative size, the presence of the contractile vacuole, nor the location of the parts in the animals which were cut to produce them. It must, therefore, in some way, be related to the nucleus. This will be considered in the following paragraphs.

The Influence of the Nucleus upon Attachment.

Dellenger (1906) made it clear that attachment always accompanies and is essential to efficient locomotion in *Amœba* and *Difflugia*. Twenty-five of my experiments on fragments of *Amœba*, in which observations were made on attachment, demonstrate quite clearly that nucleated fragments are usually continuously attached to the substratum, and that enucleated fragments are rarely attached. In these experiments some specimens were observed for only ten minutes; others for a very much longer period of time, the maximum being 72 hours. Records of attachment or unattachment in all were made at definite intervals. The following detailed description of the

results obtained in the study of one pair of segments taken from the same specimen is typical of all. In this case the parts were approximately equal in size and the nucleated part was cut from the anterior end of the amœba.

At five minutes after division both fragments were attached and moving. Three minutes later the attachment in both was broken by the sliding of a small glass rod under them. The nucleated part began immediately to send out pseudopods aimlessly. This continued for two minutes; then the segment attached and remained so for three hours—when the experiment was brought to a close. This segment was attached continuously to the substratum; it appeared to be attached usually at three different points and the behavior appeared to be normal in every respect. The enucleated segment was momentarily attached at several different times, and each time the attachment was so weak that it could not resist even the slightest jar given the table on which the experiment was made. At no time was this segment continuously attached longer than two minutes and it was never attached at more than one point at a time. There was a slow streaming motion in the protoplasm but no locomotion at all except for a short time during its first attachment and then it was very slight. The results obtained in all of the observations on the attachment of segments of *Amœba* are briefly summarized in Table I. By referring to this table it will be seen that attachment of nucleated parts to the substratum is *continuous;* that attachment of enucleated parts is *intermittent, slight* and of *short duration.*

These facts seem to indicate that the nucleus directly influences the attachment of protoplasm to the substratum and thus influences behavior.

Summary.

1. *Amœba proteus* moves regularly and smoothly by alternate formation of pseudopods on the two sides of the organism. Locomotion in segments of *Amœba* with a nucleus is of the same general character. Movement in segments without a nucleus, however, is irregular, jerky, very much slower than that in nucleated parts, and the pseudopods are not ordinarily formed regularly or alternately.

2. In a horizontal beam of light, normal specimens direct

their locomotion from the source of stimulation, *i. e.*, they orient and are negative. Parts containing a nucleus respond in the same manner; those without a nucleus, however, do not orient.

3. The rate of locomotion varies greatly in different individuals. Large specimens move more rapidly than small specimens, the rate of locomotion bearing a fairly definite ratio to the size of the specimen. The rate of locomotion in nucleated segments bears essentially the same ratio to their size as it does in normal individuals. Segments without a nucleus show very little locomotion and this is always relatively very slow and irregular.

4. The size of the parts, the contractile vacuole, and the position which the parts occupied in the intact specimens before division seem to be in no way responsible for differences in the behavior of nucleated and enucleated parts of *Amœba*.

5. The only other known respect—aside from those mentioned—in which the two parts differ concerns the nucleus. Consequently the differences in the behavior of these parts are, in all probability, in some way related to the nucleus.

6. The regulatory influence of the nucleus on behavior in *Amœba* seems to be brought about by some sort of an influence upon the attachment of the organism to the substratum.

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NOTES ON SUPERFETATION AND DEFERRED FERTILIZATION AMONG MICE.

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The phenomenon of superfetation, or the occurrence of a second conception in a mammal already pregnant, has been reported and discussed by various writers since the time of Aristotle. The latter[1] refers specifically, in this connection, only to man and the hare. Modern writers[2] have described occasional instances for several animals (man, cat, rat, sheep). On the other hand, this phenomenon appears to have been generally regarded as a biological curiosity, while its very existence has been questioned by some,[3] the facts observed being explained on other grounds. Apparently, it has been very rarely observed in the rat, for Miss King records only two cases among the more than 700 normal broods born in her stock.[4] For mice, I have thus far found no published records of supernumerary broods, though two well-known zoölogists, who have reared varieties of the house mouse in large numbers, assure me that they have encountered cases of this sort. In the absence of exact records, I am unable, however, to report these instances in detail.

In the course of several years' experience in breeding white mice, the present writer has occasionally noted the birth of a second litter, in cases where an earlier litter had been kept with the mother for some time after weaning. This was at first attributed to the precocious sexual development of the earlier male offspring, one of which was held to be the father of the later

[1] See bibliographic references.

[2] Marshall, 1910; King, 1913 (also papers cited by the latter).

[3] Schultze, 1866; Godlewski, 1914.

[4] I do not here include those cases (known as superfecundation), in which members of the same litter, derived from the same period of ovulation, are born one or two days apart. I have myself observed a few cases of this phenomenon, but shall not discuss them here.

brood. In one case of which I have record (Table II., No. 1), this may well have been the true explanation, since the second conception must have occurred when the three males which constituted the first brood were about 42 days old. That male, and even female, white mice may become sexually mature at this age I am certain from independent evidence. I have record of at least one case in which a female, mated to a male of the same brood, became pregnant at an age of about 6 weeks (between 38 and 44 days).

In one instance, however (Table I., no. 6) such an explanation was not applicable, owing to the extreme youth of the first litter, at the time of the second conception. On March 11, 1909, a female gave birth to a brood of two (1 ♂, 1 ♀). On April 8, or 28 days later, a second brood of seven was born. Since the period of gestation of this animal is normally about 20 days,[1] the mice of the first litter were scarcely more than a week old when the second lot began their development. It is needless to point out that a blind and helpless young male of this age could not have played the part of a father. Furthermore, the female had been separated from all other adults of either sex, since the day of the birth of the first brood of young, 28 days previously. Under these circumstances, to be sure, the possibility is not excluded that the second litter resulted from a copulation occurring immediately after the birth of the first. In this event, the only anomaly would be the deferred birth of the later brood of young.

Unfortunately, no further notes on this subject were made during my experiments on white mice. It is my impression that other instances similar to the foregoing were observed, but I find record of only these two cases.

It is the chief object of the present paper to furnish data relating to superfetation and deferred fertilization which have been recorded incidentally during the past two years, in the course of rearing wild mice of the genus *Peromyscus*. Unfortunately, my data are in some cases somewhat equivocal, since the experiments were not made with a view to studying these particular problems. But definite answers can none the less be given to certain questions, even if not to others.

[1] My own observations confirm those of Daniel (1910) on this point.

My stock comprises four geographical races of *Peromyscus maniculatus*, viz., *rubidus*, *sonoriensis*, and two quite distinguishable races within the assemblage generally known as "*gambeli*."

In breeding these mice, it is my general practice to place one male with three to five females. Each male is left with the respective group of females for a varying period, which ordinarily does not exceed 20 days. Record is in every case kept of the day when the male is admitted, and of the day when he is removed. Each female, when obviously pregnant, is isolated in a separate cage. The young are ordinarily left with the mother until they are 6 to 8 weeks old. At this time, they are commonly separated according to sex, the males and females being thenceforth kept apart until they are mated, according to the requirements of the work. Occasionally, this separating is not done early enough, however, and matings between brother and sister, or between mother and son, occur. The youngest mice in which I have known fertilization to take place were both 42 days old (possibly a few days younger), the next youngest pair were 55 days old or less. Because of these precocious, and therefore, unexpected conceptions, the exact date of birth of the resulting young cannot always be recorded. They were sometimes several days old when first found. Now it is among very young parents that the phenomena to be discussed seem to be commonest, as will be pointed out presently. For this reason, it happens that in all but two among the eight cases comprised in my first table the father was still present in the cage, and had access to the mother up to the time of the isolation of the latter, when her brood was first discovered. This uncertainty as to the date of the last coition somewhat complicates the interpretation of these cases, though, as will be shown, the evidence, even here, for a deferred fertilization of the ova is not thereby affected.

Before considering the phenomena presented by the tables, it will be well to make inquiry as to the normal period of gestation in *Peromyscus*. Since I have been interested only incidentally in determining this period, I have in no case restricted the mating of the parents to a single observed copulation, as has Daniel (1910), nor have I determined the exact hour of the birth of the brood, as have Long and Mark (1911). I have recorded, how-

ever, the period during which the male had access to the female, and the date of discovery of the brood. During the times when frequent births were expected, the nests were examined daily. Hence a given birth must commonly have occurred between two known observations. It has been my invariable practice to refer the birth to the day when the brood was discovered,[1] except where circumstances were such as to make it probable that the young were born a day or two earlier. In such cases, this probability is indicated in the records.

Since, in a large series of experiments, it is probable that successful insemination would occur in some cases pretty soon after the males were introduced into the cages, it seems likely that the *minimum* interval between the introduction of a male and the birth of a brood represents the actual period of gestation, at least for the individuals in question. This minimum was found to be 22 days. In all but five cases, however, out of a total of 206 broods, for which I have accurate records, the figure was greater than this. As a control, I have determined, for each series, the *maximum* interval between the removal of the male and the discovery of a brood of young. This maximum figure is also a fraction over 22 days. In the single instance recorded, a brood was known to be born as late as some time during the 23d day after the last opportunity for copulation.

From these data, therefore, we know that the period of gestation of *Peromyscus* does not, in some cases at least, *exceed* 22 days. We also know that it may be *as much as* 22 days. Accordingly, I have provisionally adopted this figure as representing the normal term for the species which I am engaged in breeding. It is likely, however, that this term is subject to some variation.

Tables I. and II. comprise two different classes of cases. In the former, the interval between the birth of the first and second litters ranges from 13 to 39 days. In all of these cases the youth of the first brood of young, at the time of the second conception, precludes the possibility that a male of the former lot was the father of the second. Even when the interval was as great as 39 days, conception must have occurred when the first lot was

[1] Mice (at least white mice) are said by Long and Mark (1911) to be more commonly born in the early morning than at any other time of day.

TABLE I.

CASES IN WHICH A MALE OF THE FIRST LITTER COULD NOT HAVE BEEN THE FATHER OF THE SECOND.

Case No.	Race.	Last Opportunity for Copulation.	Date of Birth of First Litter.	Number When First Counted.	Number Surviving Nearly or Quite to Maturity.	Interval Between First and Second Litters.	Date of Birth of Second Litter.	Number in Second Litter.
1	*gambeli* (LaJ.)	Aug. 12 (or before)	Aug. 12, 1915.	1	1 ♂	13 d. (±)	Aug. 25 (±)	3
2	*gambeli* (Berkeley)	July 30	July 30, 1915	2	none	26 d.	Aug. 25	3
3	*gambeli* (LaJ.)	Aug. 11	Aug. 11, 1915 (or before)	1	1 ♂	27 d. (±)	Sept. 7 (or before)	4
4	*gambeli* (LaJ.)	Aug. 11	Aug. 11, 1915 (or before)	1	1 ♀	27 d. (±)	Sept. 7 (or before)	5
5	*rubidus*	May 17	June 5, 1915.	2	2 ♀	28 d. (±)	July 3 (±)	3
6	*albino* (Mus)	March 11	March 11, 1909.	2	1 ♂, 1 ♀	28 d.	Apr. 8	7
7	*sonoriensis*	Aug. 12	Aug. 12, 1915 (or before)	3	3 ♂	37 d. (or more)	Sept. 18	4
8	F_1 hybrid (*sonoriensis* × *gambeli*)	Aug. 31	Aug. 28, 1915 (±)	3	2 ♂	39 d. (±)	Oct. 6 (±)	3

not over 17 days old, *i. e.*, when the young animals had just opened their eyes. At 4 and 6 days, the young are, of course, blind and helpless. Indeed, in two cases—were further proof needed—it will be noted that the first brood contained no males, while in still another, they had not yet been born.

In cases of the second class (Table II.), the first and second litters were born 63 to 81 days apart, *i. e.*, the second conception occurred when the first brood—which in every case contained a male—was 41 to 59 days old. In these cases, the possibility is by no means excluded that a member of the first litter served as the father of the second. As already stated, there is positive evidence, both in the white mouse and in *Peromyscus*, that fertilization may occur at an age of six weeks. On the other hand, such precocity is quite exceptional, so that this explanation of the cases in Table II. seems hardly more probable than that by which we must explain those in Table I., viz., fertilization by spermatozoa received from the earlier mate. In the absence of definite evidence, however, it would be idle to speculate regarding the relative probability of these two interpretations, and I shall, accordingly, exclude the cases in Table II. from most of the ensuing discussion. Nevertheless, it is worth pointing out—what is not indicated in the table—that the first births recorded in cases 3 and 4 of Table I. are the same as the second births recorded in cases 5 and 9 of Table II. If, therefore, these latter are likewise instances of deferred fertilization, we must assume for these two mothers *the delivery of three consecutive broods, after separation from their mates.* The possibility of such a surprising occurrence surely warrants more carefully controlled experiments in the future.

In the more familiar discussions of superfetation, it appears to be invariably assumed that the later fetuses owe their existence to coition occurring *during the first pregnancy.* This assumption is made by Aristotle, in the passages referred to. Marshall (1910, p. 159) says: "If ovulation takes place during pregnancy, and if, owing to the occurrence of coition the ova become fertilized, the phenomenon of superfoetation may take place—that is to say, foetuses of different ages may be present in the same uterus."

TABLE II.

CASES IN WHICH A MALE OF THE FIRST LITTER MAY HAVE BEEN THE FATHER OF THE SECOND.

Case No.	Race.	Last Opportunity for Copulation.	Date of Birth of First Litter.	Number When First Counted.	Number Surviving Nearly or Quite to Maturity.	Interval Between First and Second Litters.	Date of Birth of Second Litter.	Number in Second Litter.
1	*albino* (Mus)	?	Oct. 30, 1908	3	3 ♂	63 d. (±)	Jan. 1 (±)	?
2	*sonoriensis*	Aug. 16	Aug. 23, 1915	4	1 ♂, 3 ♀	71 d. (or less)	Nov. 2 (or before)	2
3	*sonoriensis*	Aug. 16	Aug. 22, 1915	4	2 ♂, 2 ♀	73 d.	Nov. 3	1
4	*gambeli* (LaJ.)	Aug. 16	Aug. 21, 1915	3	1 ♂	74 d.	Nov. 3	4
5	*gambeli* (LaJ.)	May 17	May 28, 1915	2	1 ♂, 1 ♀	75 d. (or less)	Aug. 11 (or before)	1
6	*sonoriensis*	?	June 1, 1914	3	1 ♂, 1 ♀	75 d.	Aug. 15	3
7	*rubidus*	May 17	May 24, 1915	2	1 ♂, 1 ♀	79 d. (or less)	Aug. 11 (or before)	3
8	*sonoriensis*	Aug. 16	Aug. 20, 1915	2	2 ♂	79 d. (or less)	Nov. 11 (or before)	4
9	*gambeli* (LaJ.)	May 17	May 22, 1915	4	1 ♂, 1 ♀	81 d. (or less)	Aug. 11 (or before)	1

Miss King (1913, p. 385) remarks that "superfœtation . . . is applied to cases in which ovulation, followed by copulation, occurred during pregnancy and led to the simultaneous development in the uterus of two sets of ova belonging to *different* periods of ovulation." In discussing the two instances observed by her in the rat she says: "The most plausible explanation for these cases seems to me to assume that the two ovaries acted independently, ovulation occurring in one ovary some little time before it took place in the other. If copulation followed each ovulation two sets of embryos would develop in the uterus simultaneously, and they would be born at different times, depending on the interval between the two periods of ovulation" (p. 390).

I believe, with the writers quoted, and in opposition to certain others, that these younger fetuses owe their origin to later periods of ovulation, which may even occur during gestation. I cannot see the necessity, however, for assuming an independent action of the two ovaries, as does Miss King. Furthermore, I regard it as highly improbable that some of the cases which I have recorded resulted from a copulation occurring at the time of the later ovulation, whether this last took place during pregnancy or after the birth of the first brood.

My own observations point, with considerable probability, to two facts: (1) *to a definite periodicity in ovulation, continuing in some cases throughout pregnancy; and* (2), *with even greater probability, to the retention by the spermatozoa of their fertilizing power for days or even weeks after reception into the uterus or fallopian tubes.*

In support of the first proposition I will point to the rather surprising numerical relations among the figures (Table I.) representing the intervals between the delivery of the two broods by the same mother. These eight numbers are all nearly or quite multiples of 13, viz:

$1 \times 13\ (\pm)$

2×13

$2 \times 13,\ (+1?)$

$2 \times 13,\ (+1?)$

$2 \times 13, (+ 2?)$

$2 \times 13, (+ 2)$

$3 \times 13, (- 2?)$

$3 \times 13, (\pm)$

It will be seen that some multiple of a quantity lying between 13 and 14 would fit every case with the exception of one, in which the figure may have been slightly less than 13. Furthermore, let us add Miss King's two cases for the rat. In one of these, the second-born brood was delivered "about 14 days" (but not more than 14 days) after the first. In the other case, the second delivery was, she says, "about 12 days" after the first. It must be mentioned, however, that this "newborn" brood was actually found 13 days after the other.

Here, then, we have altogether 3 instances in which the broods were born about 13 days apart, 5 instances in which the interval was about twice as great, and 2 in which the interval was about 3 times as great. Statistically speaking, the case is not, of course, entirely convincing, and later findings may fail to conform to the scheme here indicated. But the probability seems to be sufficiently high to warrant its provisional acceptance.

Referring to the second table, it will be found that the intervals therein recorded could not all be interpreted as multiples of any one number, though four of them are not far from being multiples of 13. Were one disposed to have recourse to a not uncommon type of argument, he could point out that the normal variability of the single intervals might reasonably be expected to lead to a cumulative divergence in the course of five or six periods. But most of us would be reluctant to draw any conclusions from this second set of figures.

Regarding the existence of a definite periodicity in the ovulation of mice, there appears to be some difference of opinion. Most recent observers seem to agree that ovulation occurs independently of coition in the mouse and rat. Sobotta (1895), Kirkham (1910, 1913), and Long and Mark (1911) hold that it takes place not long after parturition. According to the latter authors the interval between parturition and the ensuing ovula-

tion ranges from 14½ to 28½ hours. Their observations have thrown little light, however, upon the periodicity of ovulation at other times, though they do not seem to believe that this is governed by any great regularity. Sobotta (1895) states that ovulation recurs 21 days after parturition, basing his conclusions upon the examination of several females. He regards as improbable the occurrence of intermediate periods, at intervals smaller than this.

On the other hand, it is stated by Heape (1900) that "the usual length of the diœstrous cycle for rodents is ten to twenty days," that of the rat and mouse (in England) being given as approximately ten days (p. 26). Since it is also stated (Marshall, p. 135) that "in the mouse, the rat, and the guinea-pig, ovulation occurs spontaneously during 'heat,' and generally, if not invariably, during œstrus," it would follow that in these animals, ovulation likewise occurs at intervals of about ten days. This figure approximates much more nearly than Sobotta's to the thirteen-day periods indicated by my observations. Presumably, pregnancy would ordinarily interrupt these periods of ovulation, and initiate a new cycle at its close, though the phenomena of superfetation and deferred fertilization are pretty good evidence that this is not necessarily the case.

If, as seems probable from my data, the second brood of offspring resulted from a later period of ovulation, during or after the first pregnancy, we must suppose that the spermatozoa were retained in an active condition for many days and in some cases even for weeks. Fertilization was deferred until the arrival of viable eggs in the fallopian tubes or the uterus. If we consider the intervals (Table I.) between the last possible opportunity for copulation and the birth of the second litter, we have:[1] no. 1: 13 days ±; no. 2: 26 days; no. 3: 27 days; no. 4: 27 days; no. 5: 47 days (±); no. 6: 28 days; no. 7: 37 days; no. 8: 36 days.

Thus in only a single case (the first) could copulation have occurred as late as 22 days (*i. e.*, the normal period of gestation) before the birth of the second brood. In case no. 5, if we suppose

[1] In all these cases except no. 5 and perhaps no. 1, the first brood was born unexpectedly, and it is likely that the male was still in the cage at the time of delivery.

that the fetuses developed at the normal rate, at least one spermatozoön must have retained its fertilizing power for 25 days or more. In cases 7 and 8, even if we admit the probability here of coition *after* parturition, the spermatozoa must have remained alive for 15 and 14 days respectively.

An alternative hypothesis would be to assume that *gestation* rather than fertilization had been retarded in these cases. Thus Schultze (1866), while not denying the theoretical possibility of superfetation, explains all of the reported cases in man on the basis of either the death or the retardation of the less advanced fetus. This would also seem to be the opinion of Godlewski (1914), who, after pointing out what he believes to be the physiological impossibility of this process, makes the following dogmatic assertion: "Wir müssen demnach annehmen, dass die Eier, welche den Mehrgeburten den Ursprung geben, *gleichzeitig* befruchtet werden" (781). On this assumption, we should have to suppose that, at least in cases 1 and 5, the ova which gave rise to the second brood had been liberated and fertilized simultaneously with those which gave rise to the first. In the remaining cases, on the other hand, they may have been liberated and fertilized after the birth of the first brood.

Now Daniel (1910) has shown for white mice and Miss King (1913) for white rats that parturition may be considerably deferred when the mother is still suckling an earlier brood. This retardation was found, in some cases, to be as much as 10 days for the mouse and 12 days for the rat. Both of these writers assume that fertilization ensues fairly promptly after successful insemination, and that the delay in parturition is due entirely to a prolongation of embryonic development.

If we attempt to apply this interpretation to some of the present examples, we are led into difficulties even greater than those which confront the hypothesis of deferred fertilization. For example, in case 5 (Table I.), the last opportunity for copulation was 19 days before the birth of the first litter, and about 47 days before the birth of the second. Even on the assumption that the fertilization of the second lot of eggs actually took place as late as this last day, the period of gestation was prolonged by about 25 days, *i. e.*, the normal term must have

been more than doubled. Now the first brood consisted of only two individuals, so that any retarding effect due to lactation would have been almost at a minimum. Daniel found that this amounted to only about a day for each young mouse suckled.[1]

If it be contended that the retardation probably resulted from the *gestation* of the first litter rather than their subsequent nursing, it is relevant to point out that this species of *Peromyscus* may give birth to 5, 6, or even 7 young, with little, if any, prolongation of the period of gestation beyond the normal.[2] Again, in cases 7 and 8, the birth of the second brood occurred 37 and 39 days respectively after the first. In both of these cases it is possible that copulation occurred during or shortly after the first pregnancy, since the male was not removed until after the delivery of the first brood. Thus, at the least, we must assume that the second gestation was prolonged more than two weeks, owing to the suckling by the mother of her three earlier young.

To me it seems much more credible that the cases here discussed resulted from the fertilization of recently liberated eggs by spermatozoa which had been received many days previously. I do not think it helps us at all to assume that the term of development, after fertilization, was prolonged to any such extent as we should here have to suppose. Nor do I believe that it is necessary to assume a *second* copulation to account for the later brood, though this possibility is not excluded in my experiments. To refer again to case 5, of my first table, the last opportunity for copulation was 19 days before the birth of the first brood, *i. e.*, only 3 days after the conception which initiated the development of this brood. Is it not as easy to admit the retention of living spermatozoa from this first effective copulation as it is to admit their retention from another copulation occurring only three days later?

At first glance, it is not clear why these cases of deferred fertilization should, in every instance, have followed a previous pregnancy. In other words, why should not this phenomenon

[1] For rats, indeed, Miss King found that retardation was not certain to follow unless more than five young were suckled.

[2] One brood of 6 was born 23 days after the earliest possible fertilization, while one brood of 6 and one of 7 were born 24 days after the earliest possible fertilization.

have appeared in females which had earlier merely undergone insemination, without a normal conception having first ensued? As a matter of fact, I have never found an instance, among all the births recorded, in which a first brood[1] is known to have been born more than 22 days after the last possible opportunity for coition. Why should not the spermatozoa have occasionally been held in reserve for a period of ovulation occurring some time after this last opportunity for copulation? In reply, I can only point out that coition probably occurs only when the female is in a condition of "heat." If, on the advent of the first "heat" period after the introduction of the male, fertilization did not follow insemination, it would, in a large proportion of cases, point to a sterility (temporary, at least) on the part of one or both sexes. As a matter of fact, my records show that the great majority of the females which conceived at all did so during the first half of the sojourn of the male in the cage.[2] As stated above, only one female in the course of my experiments is known to have become pregnant as late as the last possible date of copulation.

To what degree the term *superfetation* is applicable to the cases comprised in my first table is a matter of definition. Case no. 1 (as well as the two cases described by Miss King) would doubtless be covered by the term as ordinarily understood, since one period of gestation, with little doubt, was superimposed upon another. For reasons stated, it does not seem likely, however, that two sets of developing fetuses were simultaneously present in any of the other cases. But in at least one of these instances (no. 5), the sperm which fertilized the second lot of eggs must have been received during or prior to the first pregnancy. Perhaps the term superfetation might conveniently be extended so as to cover such cases as these last. But the word is plainly inapplicable to instances in which the second effective copulation occurred *subsequently* to the delivery of the first brood.

Admitting the last named possibility for most of the cases comprised in my Table I, we none the less find in them instances

[1] *First*, as distinguished from the supernumerary broods here discussed. I do not refer necessarily to the first offspring of a given mother.

[2] This commonly covered 20 days.

of *deferred fertilization*, a phenomenon which has been little recognized among mammals. It is stated by Marshall (1910, p. 136), on the authority of various investigators, that "in certain bats copulation is performed during the autumn, whereas ovulation is postponed until the following spring, the animals in the meantime hibernating, while the spermatozoa are stored up in the uterus." There is no inherent improbability of the occurrence of parallel phenomena in rodents. The presence in the uterus of active spermatozoa long after copulation ought, however, to be directly determined for these animals.

A few additional comments seem worth while before closing these notes. It must be pointed out that, in *Peromyscus*, at least, the phenomena discussed cannot be of very rare occurrence. The seven cases in the first table which relate to deer-mice have been observed in the course of rearing about 250 broods of young. Thus nearly 3 per cent. of the litters born were followed by these deferred or supernumerary broods. Whether or not the small size of these first litters (1 to 3 young) is a matter of significance I cannot conjecture.

Furthermore, it should be stated that most of these supernumerary litters comprised normal, healthy animals, more than 80 per cent. of which survived nearly or quite to maturity. In the great majority, at least, of the cases recorded for man, one, if not both, of the fetuses has been dead or imperfectly developed when born.

Again, it is worthy of remark that in four cases out of eight the parents of these supernumerary broods were both very young mice, which had not yet been separated according to sex, while in two more cases the fathers (though not the mothers) were very young animals. Since the proportion of all my broods born of parents of such an early age is very small, it seems likely that this fact is of some significance.

Finally, the possibility suggests itself that some of the alleged instances of "telegony" which are recorded from time to time, may result from the actual retention of spermatozoa received from an earlier mate. A later copulation with a different partner might happen to coincide with a conception in which the earlier insemination was really the effective one.

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FURTHER DEVELOPMENTS IN OVARIOTOMIZED FOWL.

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In several castrated Brown Leghorn females certain developments of particular interest have taken place. These developments relate not only to the plumage and other external characters but also to certain structures associated with the reproductive organs. In brief, these individuals developed male plumage and other male characters. After a time, however, certain changes in the plumage of some individuals took place, best described as a change to or toward the female type, as the case might be. Still later, the plumage changed again to or towards the male type. Usually, the development of female plumage in poullards is to be referred to a regeneration of the ovary but an examination showed that no regeneration had occurred in these individuals but that instead an organ *sui generis* had grown. A portion of the organ has been removed from each bird and sectioned. Its structure clearly is neither that of the ovary nor that of the testes. The exact nature of these organs cannot be determined at present, although their structure suggests that they have some relation to the epididymis. On the right side in normal females, both ducks and fowl, a bit of tissue is sometimes found on the spot corresponding to the site of the ovary on the other side. From this body a strand can sometimes be traced posteriorly. While this body has some resemblances histologically to that of the organs developed in the castrated females, it is impossible to assert that the latter results from the hypertrophy of the former, even though there are many other reasons for drawing this conclusion. To demonstrate the assumed relationship between the structures will require a considerable series of stages which are not at present available and whose collection will require some time.

The history of these cases may now be considered in detail.

The first is that of the pullet described in the *American Naturalist*, Volume XLVII., 1913. The chick had been completely castrated when three weeks of age. In due course of time, the bird developed a good male's plumage with large comb and spurs. However, there were a number of feathers in the dorsal region which were shaped and stippled like those of the hen but rather different in color. (Fig. 3, *b*, *American Naturalist.*) With the coming of the moult it was found that the new feathers were essentially like the old. That is, the plumage retained its intermediate character. The bird was then killed and dissected. The conditions found were so remarkable that it was thought best to await confirmatory data before publishing. On either side, at the level of the adrenals was an organ which had the appearance of a small testis, though divided into several lobes. Histologically, however, it was very different. Leading posteriorly from each of these structures to the cloaca was a yellowish white strand (cord, duct) resembling an immature vas deferens. The *left oviduct* in an infantile condition was present.

The presence of the bodies described for 1196 appears to be more usual in ovariotomized fowl because such organs, with one possible exception, have not been found among the eight or ten ovariotomized ducks that have been opened or autopsied at various times, though found in all fowl thus far examined. Both species have ranged from a few months to three years of age, all but one at least a year old. Evidently the possibility of the development of the bodies in question rests upon some genetic basis. Nor are they necessary for the assumption of male characters by the ovariotomized female, since the ducks have developed as good male plumage as the fowl.

Perhaps the question will be raised regarding the possibility that all these individuals were really males. It is desirable to consider this phase of the matter in some detail. There are two general possibilities of error—first, a possibility that an error was made in identifying the sex of the individual at the time of the operation; an error however, that would be equivalent to one made in identifying the sexes of domestic poultry by their plumage. The gonads of the male and female are quite unlike, even before hatching time (cf. Thompson, *Arch. Ent.*,

1911). The *single* ovary is a flat sheet of tissue, roughly triangular in outline, found on the left side only. The *two* testes are each cigar-shaped.

The second possibility is that a clerical error was made. It should be needless to say that great care is taken to avoid this sort of mistake. However, there are several checks on mistakes of either sort. First, in ovariotomy an incision is made on the left side only, never on the right. In the extremely rare event of there being an ovary on the right side its presence would remain unknown until much later. The right side is never examined at the time of operation. Therefore, if a supposed female were really a male, the presence of the right testis would bring the mistake to light in due course of time. Second, an infantile oviduct has always been found in the cases autopsied. These oviducts are not a vague strand of tissue but on the contrary are in about the same condition as a four or five month pullet's before the ovary has begun to enlarge preparatory to laying. Third, there are a number of peculiarities about the castrated females that differentiate them from males both normal and castrated. These are fully considered in another place.[1] Finally, it may be noted that the number of instances on record is fairly large. During the past year a flock of fifteen ovariotomized ducks, besides several ovariotomized fowl, have been maintained at this station.

The second individual for consideration is a rose-combed bird of a different strain of Brown Leghorns which was castrated as a four-weeks-old chick in 1914. This bird, number 3840, developed a beautiful male plumage in due course of time, except that for some reason the development of the tail was imperfect. The main tail feathers were present but had an injured appearance. Number 1196, described above, likewise had the dorsal portion of the uropygium missing, which gave her a bob-tailed appearance. Number 3840 also had the same bob-tailed appearance in 1914, though the feathers were actually present, but lost it with the new feathers in the autumn of 1915. The comb and wattles developed more slowly than those of the normal male, but by late spring they had become large and male-like. The spurs

[1]Paper in press.

also were fully developed. When the bird moulted (1915) the new feathers were those of the *female* throughout. As is frequently the case, the bird moulted piece-meal, both kinds of feathers co-existing at one time. Gradually, however, the male feathers were replaced by feathers that could not be distinguished from those of the female. When it is recalled that the Brown Leghorns are practically identical in color with the Jungle fowl, the change can readily be appreciated. The bird was not killed as it was desired to keep her for further observation, but instead she was opened on each side. Organs roughly similar to those described for 1196 were noted. On the right side a strand of tissue was traced posteriorly for a few millimeters but no strand could be identified on the left. No trace of anything resembling normal ovarian tissue was found. As the location of the incision was unfavorable for finding the oviduct, this was not attempted. A bit of each of the organs were removed and sectioned. The histological findings were like those described below.

A short time after the operation it was noted that the new saddle feathers that came in were male-like in that they were fairly long, and laced with bright yellow, though rather bluntly pointed at the end and the central stipe though nearly black was often sprinkled with brown to a greater degree than usual for a male. The feathers of the tail coverts particularly contained much brown. In the breast feathers, less change was noticeable, most of the feathers remaining deep reddish salmon though some of the feathers contained some black. Other parts of the bird showed feathers much like those of the female type.

The third instance is that of a bird hatched June 22, 1913, and castrated August 8, 1913. The juvenile female plumage was well developed at the time. By early winter, however, this plumage had given place to one nearly or quite identical with that of the young male. The spurs developed fully but the comb never became really male-like, but had rather the general appearance of a Leghorn female's comb when straightened up. This comb, however, never loped but was always erect, an exception not at all rare among Leghorn females. It was anticipated that when the bird moulted during the summer

the full male plumage would be assumed. Instead, a different type appeared. The feathers were shaped like those of the hen, except the tail coverts which in shape resembled those of an English Campine male. That is, they were rather longer than those of the hen, curved, and with rounded ends. The dorsal feathers were dull black with golden shafts, sometimes with a few minute brown spots on the margins. Ventrally the feathers were black.

With the moult of 1915, a further change took place in that the breast feathers were replaced with salmon-colored feathers, while there was some increase in amount of brown stippling dorsally, so that this bird, too, was very much like a female. She was opened on each side in October, 1915. The same set of organs was found as described for the preceding instances. This bird, like 3840, also changed the character of its plumage after the operation. The new breast feathers were black, while the saddle feathers were good male though not as long and pointed as is usually the case.

A fourth instance has a history somewhat different from the one just preceding, although it was a litter sister and castrated at the same time. The castration, however, proved to be incomplete in that regeneration of the ovary took place. A minute bit of the ovary must have been left and when it became sufficiently large the new feathers that developed under its influence were female. Nearly a year after the first operation, a second was made and an attempt made to remove the regenerated ovary. Apparently it was successful for the bird soon after began again to assume male characters. The spurs became long but the comb has always remained hen-like, though erect. The present plumage is a mixture of male and female characters. The breast feathers are almost black but contain a little salmon in small patches. The dorsal feathers are not much longer, if any, than those of the normal female but they are triangular at the apex and have a margin of golden bristles. The centers, however, are *female* colored. An examination of the right side in October, 1915, showed a body similar to those described but rather larger. The left side seen from the right appeared to be empty but when an attempt was made to open the bird on the left side, un-

expected difficulties were met, so that it was deemed desirable to proceed no further.

There remain two other birds to be considered. Though both were of hybrid origin their plumage was that of a typical Brown Leghorn. At the time they were castrated, the female juvenile plumage was well developed. After castration both developed spurs and a perfect male coat of plumage but their combs remained small.

Number 4290 was killed November 25, 1914. The same sort of organs on the site of the gonads were noted again. The other, 4471, is still alive and in *perfect male plumage*. In the summer of 1913, a female-like plumage developed, followed in the fall by a return to the male plumage. In 1915 no change in plumage color took place. When opened, October 11, 1915, the same sort of organs were found. Thus, in all but possibly one instance there has been a development of glandular material on *both* sides. The exception relates to the individual that was examined on only one side. It seems probable that though numbers 3840 and 2087 were operated on just prior to their return to the male condition the operation as such had nothing to do with the results secured but rather that a change to this plumage would have taken place as in the case of 4471. It is of course possible that the operations accelerated a change about to take place. It is quite possible, too, that the changes in plumage are cyclic in nature, like the occurrence of the summer plumage in the normal drake.

Because it is desired to keep the birds alive for further observations, the structure of the organs cannot at present be given in detail. It was thought at first that a small piece would suffice for a determination of its structure, but the material thus far examined indicates that the study will have to be made from the standpoint of the organ as a whole. Owing to lack of material, this cannot be attempted for some time to come. However, the material on hand is sufficient to indicate something of its nature. The following description is provisional: The histological findings vary from specimen to specimen and also in different parts of tissue from the same bird. The differences, however, are probably to be referred to developmental stages,

though this is by no means certain. In what is supposed to be the early stages, the cells are small with relatively little protoplasm and closely packed. There is a very weak development of connective tissue which separates the cells into poorly defined groups. Intermediate stages can be found in which the groups of cells become well defined. After the first stage, there is a considerable increase in the connective tissue which in some instances appears to produce smooth, refractive rods or strands. Stages have been noted in which the central cells separate somewhat after the manner of thyroid tissue and stain less readily. These appear to be degenerating. Later the marginal cells also break down so that small open spaces may be found lined mostly by connective tissue. They in turn appear to coalesce by the breaking down of neighboring walls, so that large open spaces appear in the tissue, perhaps homologous with the vesicles filled with a straw-colored fluid observed macroscopically in some instances. In one part of the organ a group of tubules lined with a single layer of square cells has been noted. These appear to be of a different character from the spaces described above. They may contain a finely granular but otherwise amorphic substance.

It is rather probable that the present organ is the result of a development of the Wolfian body. This view, however, is merely a working hypothesis.

Should further investigation confirm this view, it may throw light on the structures described by various observers in cock-feathered females. These bodies are very likely identical with the bodies found in castrated females. Certainly the presence of true seminal tubules or spermatozoa together with ova must be demonstrated before similar individuals found in nature containing these organs can be designated as hermaphrodites.

An explanation of the plumage changes is mainly a matter of surmise. At first, one is inclined to lay the changes at the door of the new organ but since they do not appear in all individuals which have developed the organs, it is evident that if the organs are concerned with the changes there must be some change in the activities of the organs either preceding the changes in plumage or accompanying them.

Among ducks it has been noted that a similar change in plumage is associated with the testes in the *male*, although the organs described have not been found in several castrated female ducks which have been examined. The *ovariotomized* duck may or may not undergo a change in plumage, corresponding to that of the male. Those that undergo such a change have returned in due course of time to the breeding plumage. Thus their temporary plumage is like that of the summer plumage of the male.

The change of plumage in the ovariotomized fowl may be due to the release of a mechanism for changing the plumage but which has been hidden or rendered latent in some way during the phylogeny of the race or as a result of domestication. In this connection it may be noted that laying hens give evidence of a summer moult. This moult is rarely complete and is evidenced usually by the shedding of a comparatively small number of feathers.

THE BEHAVIOR OF THE ACCESSORY CHROMOSOMES AND OF THE CHROMATOID BODY IN THE SPERMATOGENESIS OF THE RABBIT.[1]

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The following studies resulted from a series of experiments to determine the effect of lead poisoning upon the germ-cells of the male rabbit as indicated by his offspring. My intention originally was to attempt to determine the manner in which the lead-poisoning affected the normal mitosis. The problem of the normal mitosis in itself proved to be so large that the study of the effect of lead-poisoning had to be postponed to a future time.

The following work was done under the direction of Dr. M. F. Guyer, to whom the writer is very much indebted for many valuable criticisms and kindly help. The writer is also indebted to the kindness of Professor L. J. Cole for aid given in getting the necessary material for this study.

All of the rabbit testes used were from animals raised at the barns of the Department of Experimental Breeding of the University of Wisconsin. These males were chosen from the normal stock resulting from the double matings described by Cole and Bachhuber (1914).

A fairly successful fixing reagent was found in Flemming's strong. This method brought out the chromosomal and cytoplasmic structures better than Gilson's, Zenker's, Hermann's and possibly Bouin's fixative. Bouin's gave good results in the study of the accessory chromosomes and the chromatoid body. A new method reported to be in use in McClung's laboratory was also tried with considerable success. This fixative employs urea as a means of more rapid penetration. To one hundred cubic centimeters of Bouin's, made up of seventy-five parts of

[1] Papers from the Department of Experimental Breeding, Wisconsin Agricultural Experiment Station, No. 6. Published with the approval of the Director of the Station.

aqueous picric acid with fifteen parts formalin and ten parts glacial acetic acid, were added one and one-half grams chromic acid and three grams of urea. The solution was then heated to thirty-seven degrees and the tissue added. This fixative made the chromosomes stand out somewhat better and clearer than any of the other fluids.

The sections were cut from four to fifteen micra in thickness and stained in (1) Delafield's hæmatoxylin and eosin, (2) Haidenhain's iron-hæmatoxylin and acid fuchsin or orange G, (3) safranin with gentian violet or lichtgrün. Method (2) proved to be the most successful and gave some very excellent results. This method was most valuable in bringing out the detailed structures, especially the chromatoid body. Cytoplasmic structures were as a rule satisfactorily stained in Flemming's triple stain.

Smear preparations were also made. These were fixed in Flemming's strong or in Bouin's fluid. Haidenhain's iron-hæmatoxylin gave the most satisfactory result when counterstained with lichtgrün.

Mammalian spermatogenesis seems to offer greater difficulties for study than any other form. This is due to the impossibility of securing by means of existing reagents and methods proper fixation. In nearly all preparations it has been found that the chromatic structures have a tendency to mass so that individual details are lost. The chromosomes in the rabbit have this to a very marked degree. The various stages in spermatogenesis were numerous enough, but a great many stages had to be examined in order to find those in which chromosome counts were possible.

N. M. Stevens (1911) in the spermatogenesis of the guinea-pig found that material "very unfavorable and the results are not so satisfactory as are desired." Montgomery (1912) in his work with the human spermatogenesis has to say practically the same thing, "the fixation was not as excellent as might be desired." Wodsedalek (1913), however, seems to have encountered less of the massing of chromosomes in the spermatogenesis of the pig than is present in other mammalian forms.

In both the rat and the guinea-pig, and later in the bull, the

writer has found the same condition, although to a lesser degree than in the rabbit, namely, the strong tendency of the parts within the nucleus to mass, hiding many of the individual structures, and leaving much to be desired in the form of improved methods. In the rabbit, the chromosomes always agglutinated, permitting but few chromosomal counts. Because of this, the only stages in which chromosomal counts were possible with any degree of accuracy were the primary spermatocytes. In later stages it was decidedly more difficult to find stages in which counts were possible.

In the rabbit, the structures to be followed more readily are, first, the two accesory chromosomes and second, the chromatoid body. After the spermatogonial stages, the accessory chromosomes could nearly always be identified and rather easily traced. The chromatoid body, similar to that described by Wilson (1913), whose origin could not be determined, can be easily followed, beginning with the primary spermatocytes, and can even be identified when it is cast off from the transforming spermatosomes.

The other structures displayed in the spermatogenesis of the rabbit, and the various processes connected with them, do not differ materially from the corresponding stages described for other forms. In the following pages, the process of spermatogenesis, in so far as it could be determined, will be taken up in the order of the successive stages of spermatogonia, primary and secondary spermatocytes, spermatosomes, and the fully developed spermatozoa.

1. Zones of Proliferation.

Throughout all the tubules of the testes, there appear small areas in which active proliferation reveals almost every stage of spermatogenesis. These areas are not, however, confined to any particular section or part of the tubule. All indications of the active zones go to show that a certain area may be active for a period, then halt in its activity while a nearby area becomes active in spermatogenesis. There are thus, scattered throughout each of the tubules, areas of active proliferation and areas of rest. In each of these areas of activity usually there

may be seen the ordinary arrangement of the spermatogonial cells on the outer margin with the primary spermatocytes adjacent to them. Further towards the center lie the secondary spermatocytes while the spermatids, the transforming spermatosomes, and the fully developed spermatozoa lie in the central cavity.

2. Spermatogonial Stages.

It has been difficult to obtain satisfactory preparations of the spermatogonial cells showing all of the essential structures, chromatic and achromatic. Nutritive cells were also comparatively scarce although a few may have been identified (Fig. 1). They are very similar to the resting stage of the spermatogonia with which they may easily be confused and their identity is never certain. The nuclei of these nutritive cells usually appear oval or irregular in shape and contain masses of chromatin scattered throughout This close resemblance to the nucleus of the resting spermatogonia makes it not at all improbable that the nutritive cells, if they are such, may be derived from the spermatogonia. Montgomery (1912) has found this to be true in man. He was able to trace directly the formation of the Sertoli cells from the spermatogonia by the presence of a rod-like body in the cytoplasm, which was, according to him, an invariable indication of the Sertoli cells. Hegner (1914) takes the view that the Sertoli cells arise from the primordial germ-cells. This may also be true in the rabbit although there is little direct evidence to support either view. The similarity of the Sertoli cells and the spermatogonia and the relative number of these cells affords the only evidence.

During the earliest prophases of the spermatogonial stage (Figs. 2, 3) the nucleus is somewhat elongated and irregular as a rule, and contains two or more large, spherical karyosomes. These may be the two accessory elements which can be traced very accurately after the formation of the primary spermatocytes. Small linin threads, somewhat granular in appearance, radiate out towards the periphery of the nuclear wall (Figs. 4, 5). Slender fibrillæ extend throughout the cytoplasm (Fig. 2). Between these are small areas of a granular appearing substance. The centrosome and the other cytoplasmic structures cannot be

identified although some of the more granular parts of the cytoplasm may include the centrosome and the chromatoid body. This is however doubtful because the staining reactions given by these structures in the primary and secondary spermatocytes ought also be given in the spermatogonia.

Only one spermatogonial stage has been found in which the chromatoid body appeared to be present (Fig. 6). While it has the characteristic appearance of this body, it is a rather doubtful case because it could not be identified in any other of the spermatogonial stages.

As development continues, dense masses of chromatin appear concentrating along the radial linin threads. These masses vary in number, but never exceed the diploid number of chromosomes, making it highly possible that these masses later transform into the univalent chromosomes. The entire nucleus stains heavily with the basic dyes, indicating a large increase of the chromatin content of the cell. In this manner, from twelve to twenty-two masses of chromatin are formed, all united by heavy linin threads. Gradually the linin threads disappear, and the chromatin masses assume a more regular appearance (Fig. 7). The nuclear wall disintegrates as the chromosomes arrange themselves in the metaphase stage (Fig. 8). The spindle fibers are very indistinct although in favorably stained sections they may be made to stand out more strongly. Because of this excess of stain, the cells which were stained heavy enough to make the spindle fibers appear more plainly were useless for the study of any of the other structures.

The chromosomes at best tend to mass together, and if not strongly destained, form a huge black mass in which nothing can be distinguished. In some of the metaphase stages it is possible to count twenty-two chromosomes (Figs. 8, 9, 10). The X and the Y elements which are shown to exist from their subsequent behavior, could not be distinguished from each other or from the remainder of the chromosomes in the spermatogonial division stage. It appears probable that the two large karyosomes present in the early spermatogonial stages may represent the accessory elements because they retain their individuality and later appear to transform directly into two

chromosomes of the spermatogonial division. At times they may be traced because of a slightly more rounded form than the other chromosomes. The chromosomes in general are elongated in shape although the tendency is towards a spherical form.

During the anaphase (Fig. 11), the divided chromosomes move toward opposite poles. As soon as the chromosomes reach the pole, they go into a resting stage, the new nuclear wall appearing immediately. The new cell walls may not appear until late in synezesis. The outlines of the new nuclei are first somewhat elongated, conforming to the rough outline of the massed chromosomes. Gradually these assume a more spherical shape, the spindle-fibers disappear, and the primary spermatocytes are ready for the growth period.

3. Early Maturation Stages.

While the number of chromosomes is not large in the rabbit, the difficulties encountered in counting chromosomes were great indeed. In all of the material, the tendency of the chromosomes to mass was, as already mentioned, very much in evidence, and the most careful tecnhique in fixing and staining did little to make the results more satisfactory. There were enough cells in which chromosome counts were possible to place the probable number as twenty-two in the spermatogonia and twelve in the primary spermatocyte.

It was found that twenty-two chromosomes went to each pole in the spermatogonial division. After synapsis has taken place, the number is twelve, showing that two of the elements remain single. These two elements are the accessory chromosomes. Thus the twenty ordinary chromosomes of the spermatogonia reduce to ten bivalents in the primary spermatocytes, while the two accessories do not undergo synapsis. As will be seen, the later behavior of these univalent chromosomes is entirely distinct from that of the others.

The early prophases of the primary spermatocytes show all but two of the chromosomes which passed to the poles in the spermatogonial division to grow irregular in shape, and finally weave out into fine strands which immediately spread throughout the nucleus (Fig. 12). All of the irregular masses spin out into

fine threads constituting the leptotene threads, leaving two, still rounded chromosomes, which can from this time on be identified as the accessories. The leptotene threads, in both sections and smears, are seen to persist as independent units. Their exact number could not be determined because of their extreme length.

During this stage, the accessory elements remain as small spherical masses which can easily be identified if the mass of leptotene threads is sufficiently destained. During the process of synapsis also, the X and the Y remain unchanged, always close together, and sometimes apparently connected by thin, dark-staining threads of chromatin material (Fig. 15). No instances have been found which at this stage show the accessory elements in different portions of the cell.

In these prophases, the presence of the chromatoid body becomes definitely established. It stains similarly to the chromatic material in the iron-hæmatoxylin, the Delafield's hæmatoxylin, and in the Flemming's triple stain. Just where this body arises is still a question. As before stated, it has been found in only one spermatogonial cell and that was a rather doubtful case. In the primary spermatocytes it is absolutely constant (Figs. 16, 20, 22). In a few rare cases, two and sometimes three have been found, but these extra bodies were always very small and disappeared in later stages. The large body can be traced through the remainder of the process of spermatogenesis. Further mention of its behavior will be made later.

In the synezesis stage (Figs. 13–16), the threads drift and mass at one pole of the cell into a grouping which has apparently no established order and from which nothing definite could be determined. Occasionally the threads seem to have a parallel arrangement suggesting parasynapsis, but when they come out of the synezesis stage, considerable evidence points to a chiasma-type synapsis (Figs. 18, 19). The threads are wound around each other, making possible a division later in which each of the haploid chromosomes would be made up of alternating segments of chromatin material, from the respective leptotene threads of the conjugating pair. That the threads still retain a constancy in number seems highly probable for in favorably stained sections where their ends can be seen, the number never exceeds

double the number, minus four, of the chromosomes of the primary spermatocyte.

Previous to the pachytene stage the cells have increased but very little in size. Now they begin to grow rapidly, complete fusion occurring between the conjugating threads, and all gradually drift back to the center of the cell, there to spread slowly throughout the nucleus. The threads enlarge, become lighter in staining capacity, due perhaps to the greater volume occupied by the pachytene thread, and finally form the large spireme (Figs. 17, 20, 21).

This stage of the primary spermatocytes, with the possible exception of the synezesis stage, persists longer than any other stage of the spermatogenesis. This conclusion is reached because these stages of the primary spermatocytes can be found in practically any portion of the tubules. Numerous as they are, it is extremely difficult to gain any knowledge of the processes in progress in them.

The accessory elements still retain their spherical form, usually lying closely side by side, but at this time occasionally in different portions of the cell (Figs. 17, 21). The chromatoid body enlarges and seems to become more prominent. At the same time it seems to acquire an activity in the cell although its function could not be determined.

4. Reduction Division.

The spireme of this stage in general appeared to be continuous, although some of the smears made under considerable pressure gave indications of segmentation (Fig. 23). The large spireme now condenses and soon forms the individual chromosomes. Up to this period of condensation the chromomeres stand out very distinctly (Figs. 18, 19). These condense to form the large mass of bivalent chromosomes which, because of their agglutination, usually stain so dense that the individual structure is entirely lost.

The X and Y elements, however, can nearly always be identified in these stages. As soon as the bivalent chromosomes are formed, they migrate into the equatorial plate. The accessory elements are nearly always a little to one side of this plate

(Fig. 23), usually close together. It may be that this position is constant, but the massing of the chromosomes hides the accessories (Fig. 24). In the division, the X and Y chromosomes move towards the poles long before the division of the ordinary bivalents (Figs. 25–29). The X is thus drawn to one pole, the Y toward the other. In this manner, each of the secondary spermatocytes will contain only eleven chromosomes, ten ordinary and one accessory (Fig. 30). The ordinary chromosomes, in their bivalent condition, are approximately twice the size of the spermatogonial chromosomes.

The split in the bivalent chromosomes is rarely in evidence except immediately preceding the division, although in a few isolated cases a V-shape becomes noticeable.

When the chromosomes reach the pole the chromatoid body seems to acquire more activity. During the formation of the equatorial plate, this body migrates close to the densely crowded chromosomes (Figs. 24–27). As soon as these start moving toward the poles, the X and Y preceding, the chromatoid body moves in between the two sets of chromosomes, lying approximately midway between the two centrosomes (Fig. 31). Just before the new cell wall is formed, this body moves into the cytoplasm of one of the newly formed spermatocytes. The possibility presents itself that the chromatoid body always follows either the X or the Y elements. The two accessories are, however, so closely related in size that it has been impossible to determine whether there is any such specific association.

Occasionally, in the division of the primary spermatocytes, there is found evidence that the accessory elements undergo a precocious division. The accessories lie in the plane of division and under certain conditions, they divide, forming two X and two Y elements. The division then proceeds in the regular manner with this exception: two X and two Y chromosomes go toward the poles, again preceding the division of the ten ordinary chromosomes (Figs. 32, 33).

5. Secondary Spermatocytes.

The division of the secondary spermatocytes proceeds without any appreciable rest stage. In the telophases of the preceding

division the nucleus is somewhat crescent shaped, but soon assumes a spherical form. This stage develops no spireme or leptotene threads. The chromosomes, eleven in number, immediately migrate into the equatorial plate to form a very dense mass which at times appears almost homogeneous, so closely are the chromosomes drawn together (Fig. 34). In this division the chromosomes, in migrating toward the poles, often appear as solid black ring-like masses with apparently no segments representing the individual elements. Dense fibers seem to be interwoven in the mass of chromosomes (Figs. 35, 36). It is seldom that the chromosomes can be distinguished in these stages. The X and the Y elements also as a rule lose their identity, but occasionally they can be identified by their presence in the center of the ring, connected by chromatin strands to the ordinary chromosomes (Figs. 37, 38).

The chromatoid body appears to undergo no division in these stages, following either one or the other of the chromatin masses. We thus find that approximately one-fourth of the total spermatids contain the chromatoid body.

6. Transformation of the Spermatids.

As soon as the division is complete, a new nuclear wall is immediately formed. The chromosomes as such disintegrate but the chromatic material soon condenses at the periphery of the nucleus (Figs. 39, 40). In this manner nearly all of the chromatic material is condensed, leaving one part which in every case is found somewhere near the center of the cell (Figs. 41, 43) and which may represent the accessory element. Sometimes there are one or more smaller bodies which may be remnants of the disintegrating ordinary chromosomes because they do not persist for any length of time. The large central body can be traced through the stages of further condensation and almost to the completely developed sperm.

After all of the chromatic material has condensed, the centrosome makes its appearance and moves close to the nucleus (Fig. 41). It here gradually becomes immeshed in strands of chromatic material within a slight infolding of the periphery of the nucleus. By a continuation of this process it soon becomes

entirely surrounded by nuclear material (Figs. 41, 42, 43). The entire nucleus then migrates to one side of the cell and gradually leaves behind all, or very nearly all, of the cytoplasm, together with the chromatoid body (Figs. 43, 44, 45). At the same time the entire nucleus begins to condense and finally forms a flat plate, which has at times, however, the appearance of a convex plate (Fig. 46). In the meantime the sperm tail has been formed from the cytoplasm with the centrosome in the neck. The nucleus, now the sperm head, gradually enlarges with a resultant diminution of staining capacity. In properly stained sections and smears there can occasionally be distinguished a number of darker staining bodies one of which is usually larger than the others. The number of these approaches very nearly the number of chromosomes which went into the sperm head. It is thus possible that the chromosomes retain their individuality even in the fully developed spermatozoa (Figs. 47, 48). As the sperm head enlarges, these dark-staining bodies gradually diffuse through the entire head (Figs. 49, 50). Further enlargement brings about the complete development of the spermatozoön. The head manifests an even staining capacity, the neck shows the presence of the centrosome, while the tail takes such a light stain that it is barely visible (Fig. 51).

7. Conclusions.

1. The number of chromosomes in the spermatogonium is probably twenty-two.

2. The number in the primary spermatocytes is placed at twelve.

3. The number in the secondary spermatocytes is placed at eleven.

4. Two accessory elements, an X and a Y, are present. One-half of the spermatozoa contain the X, and the other half, the Y element.

5. A chromatoid body is present. Its function was undetermined. It underwent no division, and was finally cast off with the excess cytoplasm in the metamorphosing spermatid.

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Explanation of Plates.

All drawings made with camera lucida. Spencer 2 mm. apochromat oil immersion objective and × 16 compensating eye-piece used. All drawings ar- shown at a magnification of approximately 1,800 diameters. Drawings are accurate with reference to the nuclear material and the chromatoid body and their position in the cell; the cytoplasm, however, is represented conventionally. *S*, Sertoli cell; *C*, chromatoid body.

Plate I.

Fig. 1. Sertoli cell, showing the relation of the metamorphosing spermatosomes to the nurse cells.

Figs. 2, 3. Early stages of the spermatogonia with the two large karyosomes. Fig. 2 also shows the slender fibrillæ in the cytoplasm.

Figs. 4, 5. Later stages of the spermatogonial nuclei showing the linin threads radiating out towards the periphery of the nuclear wall.

Fig. 6. Shows the only spermatogonial cell in which a body was found which resembled the chromatoid body.

Fig. 7. In this stage a condensation of the nuclear material is taking place, forming masses which later transform directly into the chromosomes of the spermatogonia.

Figs. 8–10. Metaphase stages of the spermatogonia showing the probable twenty-two chromosomes. Fig. 10 is taken from a smear preparation.

Fig. 11. Shows the divided chromosomes passing towards the poles. The X and the Y elements cannot be identified at this stage.

Fig. 12. Prophase of the primary spermatocyte showing the chromosomes from the previous division weaving out into fine strands which immediately spread through the nucleus.

Figs. 13–15. Synezesis stages of the primary spermatocytes showing the massing of the fibers, accompanied by a slow condensation, as shown in Fig. 15. This also shows the two accessory elements, which retain their spherical shape, while the ordinary chromosomes weave out into the leptotene threads.

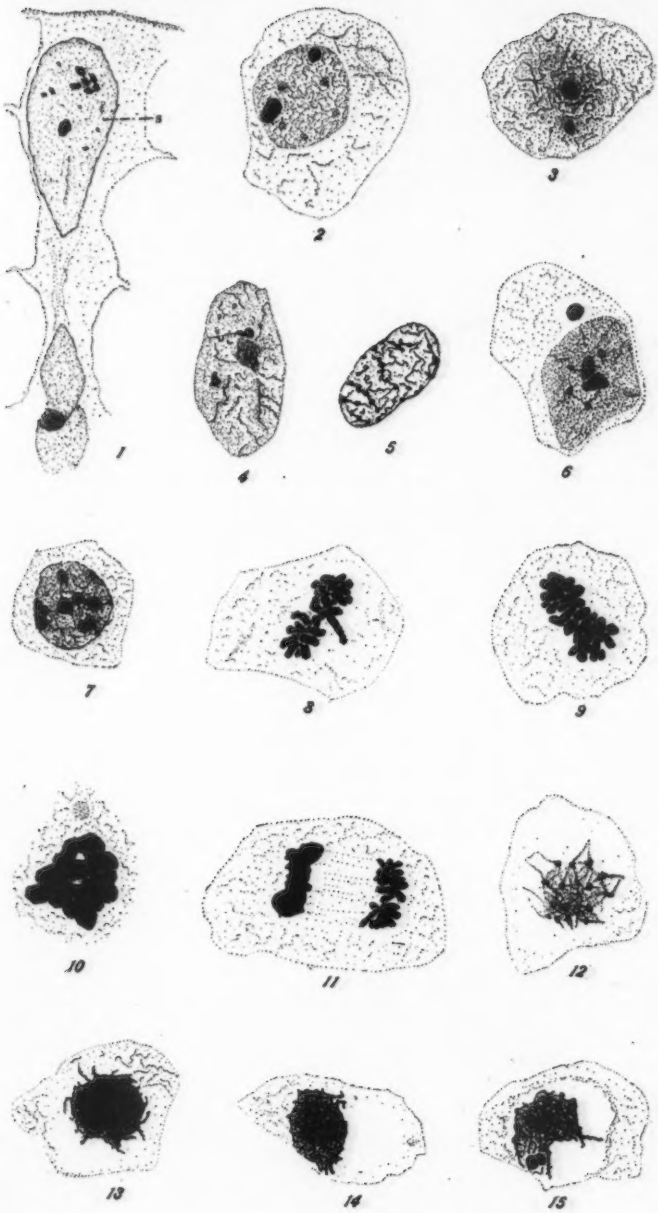

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PLATE II.

FIG. 16. Shows a further condensation of the leptotene threads, which finally results in the large spireme. This stage also shows the first appearance of the chromatoid body.

FIG. 17. The two accessory elements lie a little to one side of the condensing leptotene threads. The accessories can nearly always be identified in these stages.

FIGS. 18, 19. These stages show the leptotene threads coming out of the synezesis stage and giving evidence of a chiasmatype synapsis.

FIGS. 20, 21. The accessory elements are very conspicuous in the large spireme stages.

FIG. 22. Cell from a smear, showing evidence of a segmentation of the large spireme.

FIG. 23. The accessory elements lie a little to one side of the chromosomes massed in the equatorial plate stage, just previous to division. The chromatoid body has migrated into the same plane as the chromosomes.

FIG. 24. Shows the massing of the chromosomes, which usually hides the accessory elements. The chromatoid body is very prominent.

FIGS. 25–28. The two accessory chromosomes travel towards the poles long before the division of the ordinary chromosomes. The chromatoid body is still in the plane of the equatorial plate.

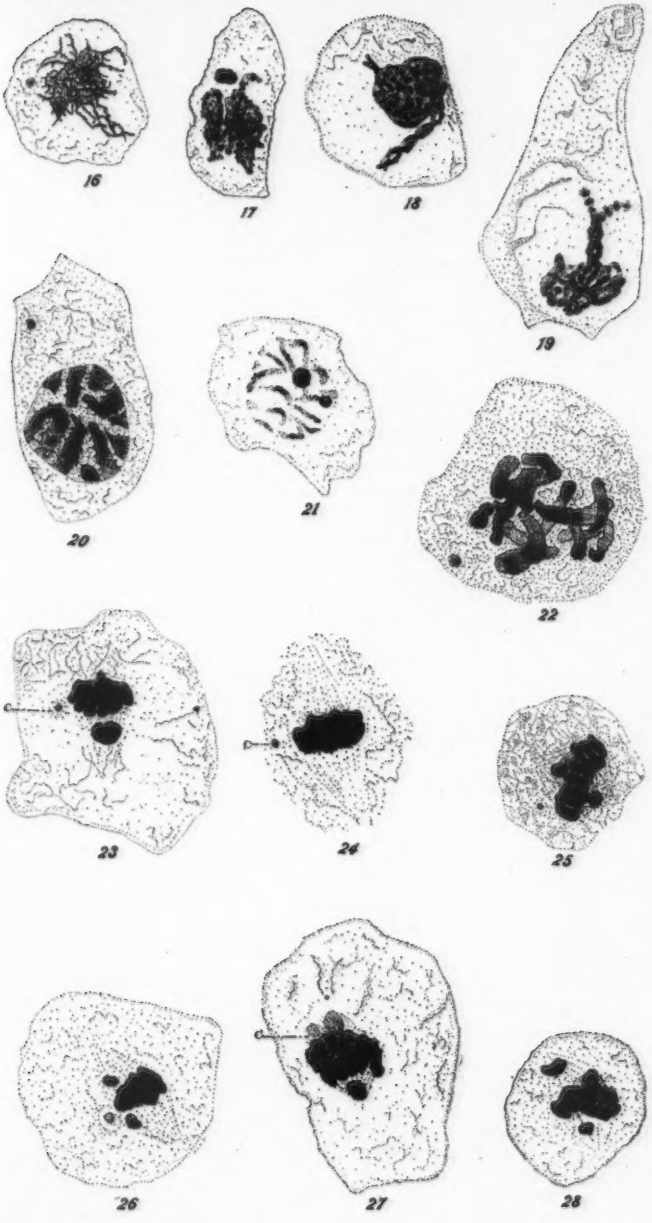

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PLATE III.

FIG. 29. Again showing the accessory elements migrating towards the poles in advance of the ordinary chromosomes.

FIG. 30. A stage following division of the chromosomes of the primary spermatocyte showing the eleven chromosomes at the pole, the accessory in the center.

FIG. 31. Shows the chromatoid body migrating in between the two sets of chromosomes immediately after division.

FIGS. 32, 33. These cells give evidence of a precocious division of the X and the Y elements. In this case, two X elements and two Y elements travel towards the poles.

FIG. 34. The chromosomes of the secondary spermatocytes have immediately lined up in the equatorial plate stage, ready for the next division.

FIG. 35. The chromosomes of the secondary spermatocytes divide and pass to the poles in ring-like masses, practically losing their identity as individual elements. This stage also shows the chromatoid body.

FIG. 36. Shows the chromosomes after reaching the poles and before formation of the spermatids. The chromatoid body is still present.

FIGS. 37, 38. Show the occasional identification of the accessory elements in the center of the closely interwoven mass of chromosomes, giving a ring-like appearance.

FIGS. 39, 40. Condensation of nuclear material at the periphery of the nuclear wall. Also shows the presence of the chromatoid body.

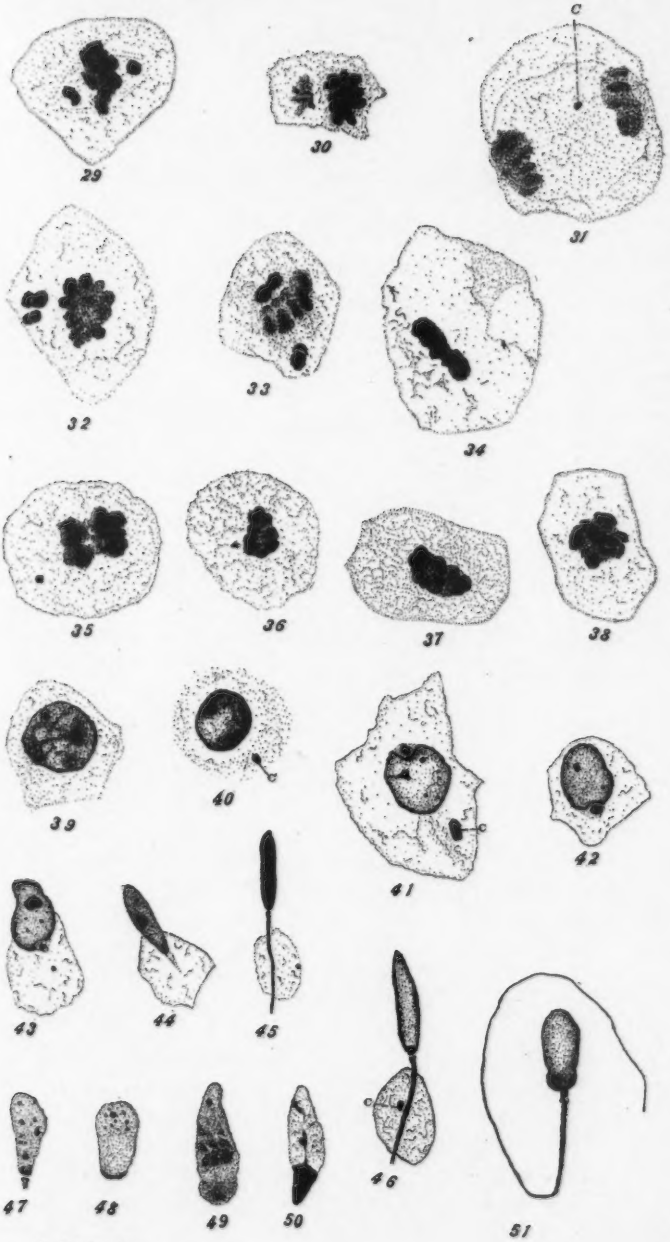
FIGS. 41–43. Further condensation of chromatin around the periphery of the nuclear wall. The centrosome is meshed in one side of the nucleus. The chromatoid body may lie in any portion of the cytoplasm and may be very irregular in shape.

FIGS. 44–46. Still further condensation, with the gradual escape of the nucleus from the excess cytoplasm. The chromatoid body is cast off with the excess cytoplasm.

FIGS. 47, 48. Show the presence of darker staining masses of chromatin material which may represent the individual chromosomes in the sperm head.

FIGS. 49, 50. The darker staining masses now diffuse through the sperm head and finally form an even-staining mass of chromatin.

FIG. 51. Fully developed spermatozoön, showing the head, the middle-piece with the centrosome, and the long, thin, lightly staining sperm tail.



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